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Short Communication

Frequency of plasmid-mediated AmpC in Enterobacteriaceae isolated in a Brazilian Teaching Hospital

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Abstract

In Brazil, the presence of plasmid-mediated AmpC (pAmpC)-producing isolates has been sporadically reported. We evaluated the frequency of pAmpC among 133 Enterobacteriaceae clinical isolates. The bla_{CMY-2}-like gene was detected in a single *Klebsiella pneumoniae* isolate. In our study, the pAmpC frequency was very low as previously reported.

Key words: plasmids, beta-lactamases, beta-lactams, Brazil.

The production of plasmid-mediated AmpC (pAmpC) enzymes conferring resistance to third-generation cephalosporins has been increasingly reported among *Escherichia coli, Klebsiella* spp., *Proteus mirabilis* and *Salmonella* spp. isolates worldwide (Philippon *et al.*, 2002). Most acquired *ampC* genes are derived from chromosomal genes, which are incorporated and mobilized by plasmids making easier their spread (Pérez-Pérez and Hanson, 2002). CMY-type enzymes are the most frequent reported pAmpC β-lactamase (Jacoby, 2009). In Brazil, the presence of pAmpC-producing isolates has been sporadically reported (Castanheira *et al.*, 2007; Pavez *et al.*, 2008). FOX-5 and CMY-2 were both detected in *E. coli* strains isolated from two distinct Brazilian geographic regions. The FOX-5 encoding gene was unexpectedly found, during the DNA sequencing of a 41-kb conjugative plasmid that harbored a *qnrA* gene and a class 1 integron with the *aadB* and *catB3* gene cassettes (Castanheira *et al.*, 2007). On the other hand, bla_{CMY-2} was detected in four carbapenem-resistant *E. coli* strains isolated successively from a single patient (Pavez *et al.*, 2008). Despite of these two reports, studies reporting the pAmpC frequency among Brazilian isolates are still scarce. Dias and colleagues (Dias *et al.*, 2008) have studied the prevalence of pAmpC among Enterobacteriaceae strains isolated from a teaching hospital in Rio de Janeiro, Brazil. In that study, the production of pAmpC beta-lactamases were not detected and the multidrug resistance phenotype observed in five *E. coli* isolates was attributed to the hyperproduction of chromosomal AmpC (Dias *et al.*, 2008). Since resistance to broad-spectrum cephalosporins has been commonly observed among Enterobacteriaceae isolated in our hospital, especially due to ESBL production, we conducted a study to evaluate the frequency of pAmpC among Enterobacteriaceae clinical isolates. We evaluated a total of 133 Enterobacteriaceae (41 *E. coli*, 05 *Klebsiella oxytoca*, 65 *Klebsiella pneumoniae*, 18 *P. mirabilis*, and 04 *Salmonella* spp.) isolates consecutively collected from bloodstream of patients, who were hospitalized at Hospital São Paulo, São Paulo, Brazil, between January and July 2006. The antimicrobial susceptibility profile was determined by agar dilution method and interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2009, 2011) recommendations. The following antimicrobial agents were tested: aztreonam, ceftazidime, cefepime, cefoxitin, ceftriaxone, ciprofloxacin and imipenem. The ESBL phenotype was detected by double disk approximation method (Jarlier *et al.*, 1988). The production of pAmpC among Enterobacteriaceae was phenotypically screened by modified tridimensional and Hodge tests (Coudron *et al.*, 2000; Lee *et al.*, 2005). The isolates showing a weak enhanced growth around the test organism streak at the intersection of the streak and zone of inhibition were classified as possessing an undetermined result by modified Hodge test. In the modified tridimensional test, results were considered undetermined, when the isolates

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showed a weak enhanced growth around the slit containing the beta-lactamase crude extract. The presence of pAmpC β-lactamases-encoding genes was carried out by multiplex polymerase chain reaction (PCR) (Pérez-Pérez and Hanson, 2002). Primers CMY-2F, 5'-ATGATGAAAAA ATCGTTATGCT-3' and CMY-2R, 5'-TTATTGCAGCT TTTCAAGAATGCG-3' were used to amplify the whole allele for sequencing of the single isolated detected as CMY-producer.

The results of this study were summarized in Tables 1 and 2. Twenty-six (20.3%) and three isolates were not susceptible to cefoxitin and imipenem (MIC_{90}, ≤ 0.5 μg/mL), respectively. Among the three imipenem-resistant isolates, only one isolate was phenotypically detected as an ESBL producer. This same isolate showed a false-positive result for pAmpC phenotypical detection by the modified Hodge test. Among the 133 isolates, 59 (44.4%) were phenotypically detected as ESBL producers. The ESBL phenotype was more frequently detected among *K. pneumoniae* (43 isolates; 72.9%) followed by *P. mirabilis* (11 isolates; 18.6%), *E. coli* (4 isolates; 6.8%), and *K. oxytoca* (1 isolate; 1.7%). The PCR method was considered as the gold standard method to calculate sensitivity and specificity rates among isolates evaluated. Both phenotypic methodologies for detection of pAmpC showed sensitivity of 100%; however, the specificity rates were different being 96% and 85% for modified tridimensional and Hodge tests, respectively. Discordant results for pAmpC phenotypic detection, *i.e.*, modified Hodge test results were different from those displayed by the modified tridimensional test were observed for 16.5% isolates (14 *K. pneumoniae*, 06 *E. coli* and 02 *P. mirabilis*). Among the undetermined (weak positive) results in the modified tridimensional and Hodge methods, 87.5% and 80% were ESBL producers, respectively. The presence of bla_{CMY-2}-like gene was observed in a single *K. pneumoniae* isolate. Nucleotide sequencing showed that the bla_{CMY-2} gene had 99% sequence identity with the plasmid-encoded bla_{CMY-2} gene first described in *K. pneumoniae* in Greece (Bauernfeind *et al.*, 1996). In the present study the frequency of ESBL-producing enterobacterial isolates (44.4%) was high, while the frequency of pAmpC-producing isolates was very low. The rates of ESBL production observed in this study were similar to other Latin American studies and higher than those reported in other geographic regions (Paterson and Bonomo, 2005). Among Enterobacteriaceae resistance to broad-spectrum cephalosporins due to pAmpC production is generally less common than ESBL production in most parts of the world. However, its frequency might be underestimated since phenotypic detection of pAmpC enzymes is not easily achieved. pAmpC-producing isolates might be screened as ESBL producers according to the former CLSI criteria but fail in the confirmatory test because pAmpC activity is not inhibited by clavulanic acid (Jacoby, 2009). Besides, clinical isolates may produce simultaneously both ESBL and pAmpC (Thomson, 2001). The resistance to cefoxitin might be used as a marker for AmpC production since ESBLs do not hydrolyze this agent. However, this phenotype may also be due to porin alterations (Thomson, 2001). Among the isolates that showed positive or undetermined results for at least one of the pAmpC phenotypic tests, 90.9% and 100% were ESBL-producing *K. pneumoniae* and -*P. mirabilis,* respectively. False detection of carbapenemase production by the Hodge test has been reported among ESBL-producing *K. pneumoniae* isolates that also had lost OmpK35 and/or OmpK-36 porins (Carvalhaes *et al.*, 2010). Furthermore, among the *E. coli* isolates phenotypically detected as AmpC producers by the Hodge test, only a single isolate was an ESBL producer. These findings might suggest overproduction of chromosomal AmpC, which is normally produced at low level in this species. CMY-variant enzymes have been reported worldwide, with the CMY-2 variant being the most prevalent and widely distributed so far (Philippon *et al.*, 2002). Given the ability of pAmpC-producing organisms to hydrolyze many β-lac-

Table 1 - Antimicrobial susceptibility among *K. pneumoniae*, *E. coli* and *P. mirabilis* isolates.

Species/(no. tested)	*K. pneumoniae* (65)				*E. coli* (41)				*P. mirabilis* (18)			
Antimicrobials tested	MIC(μg/mL)[a]		S%	R%	MIC(μg/mL)[a]		S%	R%	MIC(μg/mL)[a]		S%	R%
	MIC_{50}[b]	MIC_{90}[b]			MIC_{50}[b]	MIC_{90}[b]			MIC_{50}[b]	MIC_{90}[b]		
Aztreonam	64	256	32.3	67.7	≤ 1	16	78.0	12.1	≤ 1	8	88.9	5.6
Cefoxitin	8	32	64.6	24.6	4	8	95.1	0.0	2	8	94.4	5.6
Ceftriaxone	256	> 512	29.2	70.8	≤ 1	32	78.0	14.5	32	512	33.3	66.7
Ceftazidime	32	64	32.3	67.7	≤ 1	2	95.1	0.0	≤ 1	8	88.9	0.0
Cefepime	32	64	36.9	60.0	≤ 1	4	92.7	4.8	32	128	38.9	55.6
Imipenem	≤ 0.5	≤ 0.5	95.4	3.0	≤ 0.5	≤ 0.5	100.0	0.0	≤ 0.5	1	100.0	0.0
Ciprofloxacin	16	> 32	33.8	66.2	≤ 0.12	32	82.9	17.1	4	16	27.8	55.6

a. MIC, minimal inhibitory concentration determined by agar dilution CLSI (2009).
b. MIC_{50} and MIC_{90}, minimal inhibitory concentration that inhibited 50% and 90% of bacterial isolates tested, respectively.

Table 2 - Phenotypic detection of pAmpC phenotypes among *K. pneumoniae*, *E. coli* and *P. mirabilis* isolates.

Species/ (No. tested)		*K. pneumoniae* (65)		*E. coli* (41)		*P. mirabilis* (18)	
Detection of pAmpC phenotype		No. (ESBL phenotype)	%	No. (ESBL phenotype)	%	No. (ESBL phenotype)	%
Modified Tridimensional Test	Positive	6[a] (5)	9.2	0 (0)	0	0 (0)	0
	Negative	53 (33)	81.6	41 (4)	100	16 (9)	88.9
	Undetermined[b]	6 (5)	9.2	0 (0)	0	2 (2)	11.1
Modified Hodge Test	Positive	15[a](13)	23.1	4 (1)	9.7	0 (0)	0
	Negative	44 (24)	67.7	35 (3)	85.4	16 (9)	88.9
	Undetermined[b]	6 (6)	9.2	2 (0)	4.9	2 (2)	11.1

[a]The single strain detected as CMY-2-producer.
[b]Weak positive results.

tam antibiotics, the carbapenems are the therapeutic of choice for treating infections caused by such organisms. However, the clinical usefulness of those important antimicrobials has been jeopardized by the emergence of carbapenem-resistant *K. pneumoniae* due to pAmpC and/or ESBL producer coupled with porin loss (Philippon *et al.*, 2002) and more recently by the emergence and spread of carbapenemase producing isolates. Although reported with increasing frequency in other geographic regions, our results agreed with previous reports and pointed out that pAmpC were not frequent in our institution (Dias *et al.*, 2008). ESBL production is still the main mechanism of resistance to broad-spectrum cephalosporin among clinical isolates. Despite of the low pAmpC frequency in our hospital (data not shown), continuous surveillance is important for early detection of this mechanism of resistance and prevention of its spread since it was already detected in our environment. Moreover to reach this aim, the development of accurate phenotypic tests is urgently needed.

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